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Title: Radio Frequency Electric Fields Inactivation of *Escherichia coli* in Apple Juice Using an 80 KW Power Supply

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RADIO FREQUENCY ELECTRIC FIELDS INACTIVATION OF *ESCHERICHIA COLI* IN APPLE JUICE USING AN 80 KW POWER SUPPLY

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Abstract

The nonthermal process of radio frequency electric fields (RFEF) is relatively new and has been shown to inactivate bacteria in apple juice at moderately low temperatures. However, the process has only been developed for a flow rate of 550 ml/min. The objective of this study was to scale up the RFEF technique to greater flow rates. A novel 80 kW RFEF system was designed and assembled that processed apple juice at a flow rate of 1.4 l/min. *Escherichia coli* K12 in apple juice was exposed to an electric field strength of 20 kV/cm at a frequency of 21 kHz. Following treatment at an outlet temperature of 60 °C, the population of E. coli was reduced by 2.1 log. Increasing the electric field strength and temperature enhanced the inactivation.

Introduction

Most of the fruit juices consumed in the developed countries are pasteurized using heat. Apple juice is commonly pasteurized by heating to 77-88 °C in a heat exchanger and holding for 25-30 s. At certain conditions, the organoleptic and nutritional qualities of the juice can be damaged. Therefore, nonthermal pasteurization processes are being developed. High pressure processing and ultraviolet radiation processing have recently been commercialized, although to a limited extent. Another alternative process, that has more recently been explored, involves the use of high electric fields. In an electric field, a voltage is formed across a bacterium cell's membrane. As the voltage is increased, the opposite charges on either side of the membrane are attracted to each other with greater force and the membrane becomes thinner. At a sufficiently high voltage, pores are formed in the membrane and the cell ruptures (Zimmermann, 1986). The treatment times necessary to rupture the cells are usually less than 1 ms. Due to the electrical resistance of the juice, the electric field also raises the temperature of the juice by ohmic heating. The final temperatures generally are less than 70 °C and the juice is typically cooled using a heat exchanger within several seconds. The combination of lower time and temperature enables the juice to retain maximum fresh-like qualities.

High electric fields are produced by pumping the juice through a narrow gap between two electrodes and applying a high voltage. The high voltage can be applied by several different means. One method is to use direct current (DC); however, a disadvantage of this method is that charged particles in the juice may form a layer on the anode that would require periodic cleaning. Another disadvantage with DC is that undesirable electrolysis reactions may occur (Geveke 2003; Qin, Zhang, Barbosa-Canovas, Swanson, and Pedrow 1994). Using either bipolar waveforms or alternating current (AC) overcomes these problems. Bipolar waveforms are extensively used in pulsed electric fields processing where a charging power supply produces a high voltage and a high speed electrical switch delivers the stored energy to the electrodes. The power supply is then required to be recharged which results in pulsed processing. An AC generator continuously provides high voltage and is the source of power for radio frequency electric fields (RFEF) processing.

RFEF processing at 30 kV/cm and 20 kHz reduced the population of *Saccharomyces cerevisiae* by 3.8 log (99.984 %) at 35 °C (Geveke and Brunkhorst 2003a). RFEF processing inactivated *Escherichia coli* K12 in apple juice by 2.1 log at 21 kV/cm and 55 °C (Geveke and Brunkhorst 2003b). Raising the temperature increased inactivation. Radio frequencies of 15 and 20 kHz inactivated *E. coli* better than frequencies of 30-70 kHz. The flow rate was limited to 550 ml/min by the 4 kW RFEF power supply.

The objective of this work was to design and assemble an 80 kW RFEF system and use it to scale up the process flow rate of apple juice. The hypothesis was that the inactivation obtained at 550 ml/min could be duplicated at higher flow rates using the more powerful system.

Materials and Methods

Escherichia coli K12 substrain C600 (Fratamico 1993) was maintained on tryptose agar (Difco Laboratories, Detroit, MI) at 4 °C. The *E. coli* was cultured in brain heart infusion (Difco Laboratories) for 24 h at 37 °C. Concentrated apple juice was purchased from Tree Top (Selah, WA). The juice was diluted with water and was inoculated from the stationary phase culture to give an approximately 6 log cfu/ml population. The solution's conductivity was 2.4 mS/cm.

A RFEF experimental system that produced a maximum voltage of 9 kV at a frequency of 21.1 kHz was designed, purchased, and assembled. It consisted of an 80 kW RF power supply (Ameritherm, Scottsville, NY, model L-80) and a custom designed matching network that enabled the RF energy to be applied to a resistive load (Ameritherm). The peak voltage applied was limited to 5.0 kV in order to control the temperature rise of the apple juice.

The RFEF treatment chamber was made of Rexolite, a transparent cross-linked polystyrene copolymer (C-Lec Plastics, Philadelphia, PA). The treatment chamber was designed to converge the apple juice into a narrow flow area in order to reduce the power requirement (Geveke and Brunkhorst 2003b; Matsumoto 1991; Sensoy 1995). Juice entered and exited the Rexolite chamber through the annuli of cylindrical stainless steel electrodes (Swagelok, Solon, OH, part no. SS-400-1-OR). The electrodes were separated by a thin partition, with a channel of circular cross section through the center. The diameter and width of the channel were 0.12 cm and 0.20 cm, respectively. A 0.9 cm space between the end of each of the electrodes and the central channel prevented arcing. To increase the inactivation, the apple juice passed through two treatment chambers in series. The output of the RFEF power supply was connected to the inner electrodes between the treatment chambers and the outer electrodes were grounded. The maximum electric field strength used in the study was 25 kV/cm obtained by dividing the peak voltage, 5.0 kV, by the width of the central gap, 0.2 cm.

The supplied voltage and current to the RFEF treatment chambers were measured using an oscilloscope (Tektronix, model TDS224), current probe (Pearson Electronics, Palo Alto, CA, model 411), and a voltage divider (Ross Engineering, Campbell, CA; model VD15-8.3-A-KB-A).

The experimental system included a stainless steel feed tank and a progressing cavity pump (Moyno, Springfield, OH; model 2FG3) that supplied the apple juice to the RFEF system at a flow rate of 1.4 l/min through stainless steel tubing as shown in Figure 1. The inlet temperature to the RFEF treatment chamber was controlled using a stainless-steel heat exchanger (Madden Manufacturing, Elkhart, IN; model SC0004) and a temperature controller (Cole-Parmer, model CALL 9400).

The temperatures of the apple juice immediately before and after the RFEF treatment chambers were measured with thermocouples. The temperatures were continuously logged to a data acquisition system (Dasytec USA, Amherst, NH, Dasylab version 5.0).

The apple juice was quickly cooled after exiting the treatment chamber to less than 25 °C using a stainless-steel heat exchanger (Madden Manufacturing, model SC0004). The length of time for the juice to travel from the treatment chamber to the sample cooler was 2 s.

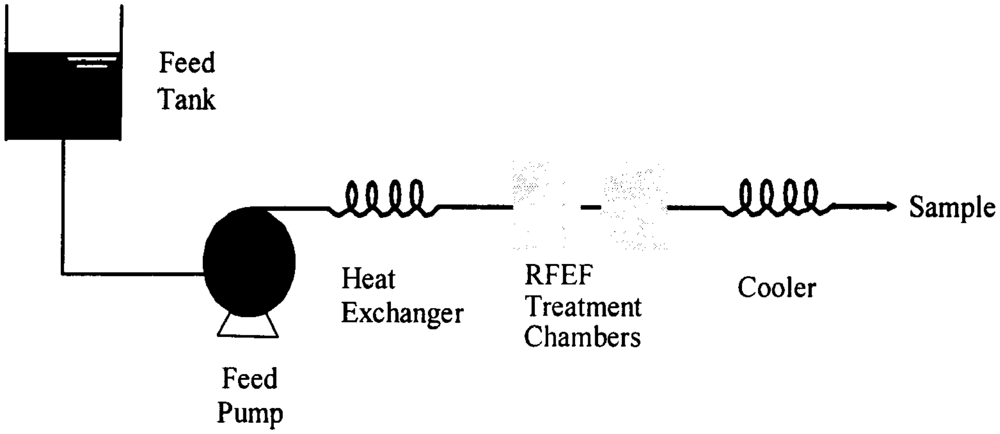

Figure 1. Schematic diagram of continuous RFEF process.

Controls were performed to determine the effect of temperature alone. In order to ensure that the control juice received the same time and temperature history as the treated juice, the high fields, converged treatment chambers were replaced with an ohmic heating chamber. The chamber consisted of two electrodes inserted into a 10.2 cm length of 0.64 mm ID plastic tubing. The ohmic heater quickly brought the juice temperature up to the desired temperature. The control juice was identically held for 2 s before cooling to less than 25 °C.

Appropriate dilutions of the product samples were plated on tryptose agar using a spiral plater (Spiral Biotech, Bethesda, MD; model Autoplate 4000) and incubated at 37 °C for 24 h. Enumerations were made with a colony counter (Spiral Biotech, model CASBA 4).

Each RFEF experiment was performed in duplicate. Results were expressed as the means of these values.

Results and Discussion

The new 80 kW RFEF system successfully inactivated *Escherichia coli* K12 in apple juice at nonthermal conditions. Whereas the previous 4 kW RFEF system had been limited to a flow rate of 0.55 l/min, the 80 kW system was capable of treating 1.4 l/min. The extent of microbial inactivation is dependent on the electric field strength and temperature.

A series of experiments were performed to determine the effects of electric field strength and temperature on inactivation, and the results are presented in Figure 2. The population of *E. coli* in apple juice was reduced by 1.4 log after being exposed to a 20 kV/cm peak electric field at a treatment chamber outlet temperature of 55 °C and a hold time of 2 sec. When the juice was ohmicly heated to the same outlet temperature, 55 °C, and held for the same time, 2 sec, the population of *E. coli* was unaffected. Applying an electric field of 20 kV/cm at 60 °C reduced *E. coli* by 2.1 log. Increasing the field strength to 25 kV/cm at 60 °C reduced *E. coli* by 2.3 log. The RFEF reduction increased to 2.9 log at 65 °C while the thermal-control reduction remained negligible. The nonthermal inactivation is believed to be due to dielectric breakdown of the cells (Zimmermann 1974). Using a 4 kW RFEF system, *Escherichia coli* K12 in apple juice was reduced by 2.1 log at 21 kV/cm, 55 °C, 4 sec hold time, and 0.55 l/min (Geveke and Brunkhorst 2003b). The results of the present study, with a uniquely designed 80 kW RFEF system, scaled up the RFEF process to 1.4 l/min.

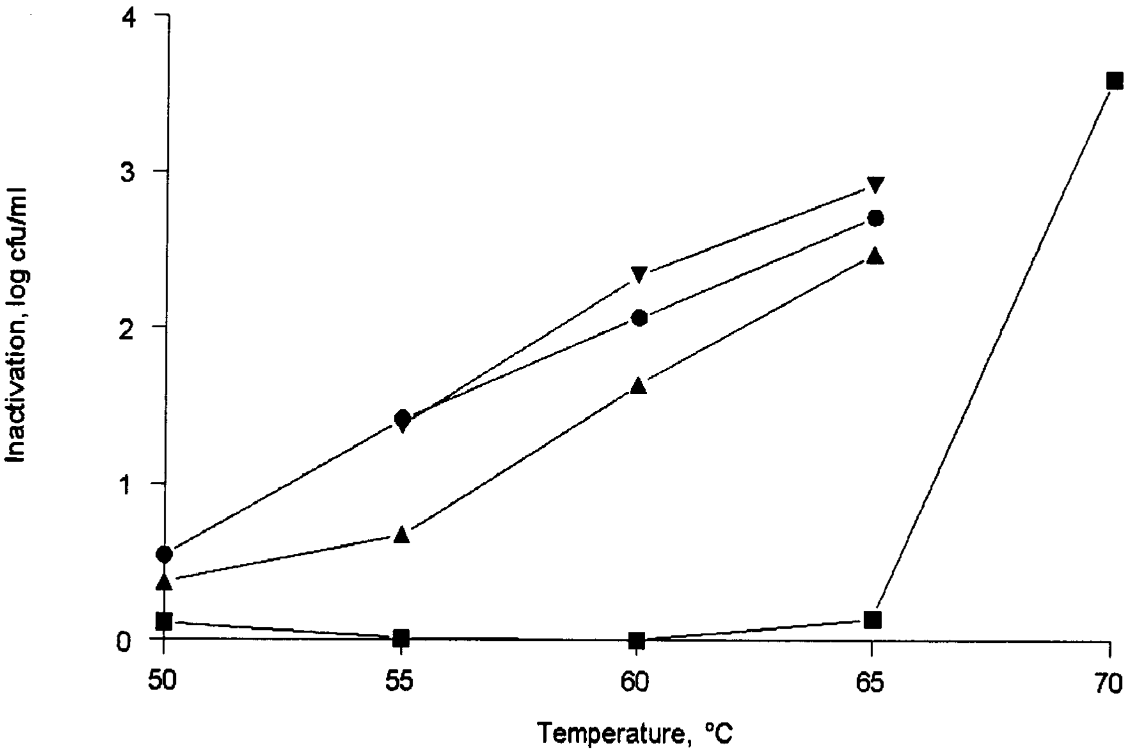

Figure 2. Effects of temperature and electric field strength on the inactivation of *E. coli* at 21.1 kHz and a 2 s hold time. ■, Control; ▲, 15 kV/cm; ●, 20 kV/cm; ▼, 25 kV/cm.

Additional studies are recommended. The RFEF process needs to be further scaled up to be of commercial interest. In addition, a 5 log reduction is desirable. This should be achievable by adding several more treatment chambers in series.

Conclusions

The radio frequency electric fields (RFEF) process was successfully scaled up from 0.55 l/min to 1.4 l/min using a novel 80 kW power supply and matching network. Nonthermal inactivation of *Escherichia coli* K12 in apple juice was obtained and is dependent upon the electric field strength and temperature.

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